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Review Article

T-type calcium channels: a potential target for the treatment of chronic pain

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CONTENTS

Abstract

The treatment of chronic pain remains a complex clinical challenge. As basic research progresses, more is being understood about the mechanisms that underlie chronic pain. Understanding such physiology and how it changes in response to nerve injury, for example, enables the identification of potential targets for analgesic therapy. This review discusses one such target: the T-type calcium channel. There are a variety of other calcium channels, such as N-, P/Q- and L-type channels. To date, research on calcium channels has proved fruitful; for example, ziconotide (Prialt®), which acts on N-type calcium channels, a target identified using animal pain models, is now available for the treatment of chronic pain.

Introduction

The upstroke of the action potential is almost exclusively generated by the influx of Na^+ ions through voltage-gated sodium channels. This influx causes the membrane potential to become less negative, which results in activation of voltage-gated calcium channels. At the start of the action potential, the intracellular Ca^{2+} concentration is much less than the extracellular Ca^{2+} concentration, and thus, opening of voltage-gated calcium channels results in an influx of Ca^{2+} into the cell. Repolarization of the cell then occurs when the efflux of K^+ ions from the cell surpasses the influx of Ca^{2+} into the cell.

The transmission of painful messages, or nociceptive transmission, from the periphery to the spinal cord is encoded in action potentials propagated by Aδ- and C-fibers. Thus, Na^+, K^+ and Ca^{2+} channels all play a role in nociceptive transmission, and are therefore potential targets for analgesic drugs. Furthermore, calcium is a crucial second messenger for intracellular signaling in neurons and neurotransmitter release. In summary, Ca^{2+} channels supply a key relationship between neuronal hyperexcitability (a major problem in chronic pain states) and synaptic transmission.

Several calcium channel subtypes have been identified: high-voltage-activated (HVA) N-, L-, P/Q- and R-type channels and the low-voltage-activated (LVA) T-type channel. Originally, L-type channels were so called because they have a large single-channel conductance and a long open time. In contrast, T-type channels acquired their name from their tiny single-channel conductance and transient open time. N-type initially referred to neither L-type nor T-type (1), but these channels are also predominantly expressed in neurons. P-type channels were termed such because of their expression in cerebellar Purkinje neurons.

In general, native calcium channels consist of a main α1 subunit and ancillary α2-δ and β subunits. Skeletal L-type channels have an additional subunit (γ). The α1 subunit consists of 4 homologous transmembrane regions (each comprised of 6 transmembrane domains) connected by cytoplasmic links. This subunit gives rise to the conduction pore and also the voltage-sensing and -gating properties of the channel (2, 3). Thus, the α1 subunit appears to dictate the main properties of the channel, whereas the ancillary subunits can modulate channel functions, *e.g.*, gabapentin binding to the α2-δ subunit. To date, 10 different genes that encode the α1 subunit have been identified and the classification of calcium channels is based upon this (see Table I).

Since the clinical analgesic efficacy of drugs which interact with calcium channels, such as gabapentin (Neurontin®) and ziconotide (Prialt®), was demonstrated, more attention is now being focused on the role of calcium channels in chronic pain. This review will focus on the

Table I: Voltage-gated calcium channel α1 subunits (adapted from Ref. 3).

α1 subunit	Splice variant	Type of current	Primary tissue expression
$Ca_V1.1$		L-type	Skeletal muscle
$Ca_V1.2$	$Ca_V1.2a$	L-type	Heart
	$Ca_V1.2b$	L-type	Smooth muscle
	$Ca_V1.2c$	L-type	Heart, pituitary, adrenal gland, brain
$Ca_V1.3$		L-type	Pancreas, ovary, kidney, cochlea, brain
$Ca_V1.4$		L-type	Retina
$Ca_V2.1$	$Ca_V2.1a$	P/Q-type	Cochlea, pituitary, brain
	$Ca_V2.1b$	P/Q-type	Cochlea, pituitary, brain
$Ca_V2.2$	$Ca_V2.2a$	N-type	Nervous system, brain
	$Ca_V2.2b$	N-type	Nervous system, brain
$Ca_V2.3$	$Ca_V2.3a$	R-type	Heart, cochlea, pituitary, retina, brain
	$Ca_V2.3b$	R-type	Cochlea, retina, brain
$Ca_V3.1$	$Ca_V3.1a$	T-type	Nervous system, brain
$Ca_V3.2$	$Ca_V3.2a$	T-type	Heart, liver, kidney, brain
$Ca_V3.3$	$Ca_V3.3a$	T-type	Brain

role of T-type calcium channels in chronic pain, summarizing the evidence reported to date to indicate that blockade of T-type calcium channels could provide a novel treatment for this condition.

Cloning and distribution of T-type calcium channels

Three α1 subunit genes have been identified that express T-type calcium channels: $Ca_V3.1$, $Ca_V3.2$ and $Ca_V3.3$. The identification of these calcium channel α1 subunits was predominantly the result of several studies performed by two laboratories. Perez-Reyes and colleagues first cloned the $Ca_V3.1$ gene from rat brain using a human expressed sequence tag (EST) clone (4). Employing this human EST clone further, they cloned the $Ca_V3.2$ gene by screening a human heart library (5). Using a different human EST clone, this laboratory screened the rat brain and identified the rat $Ca_V3.3$ gene (6). Complementary to these efforts, the work of Monteil and colleagues resulted in the cloning of the human forms of $Ca_V3.1$ (7) and $Ca_V3.3$ (8).

Table I lists the tissues in which the T-type calcium channel isoforms are primarily expressed. The distribution of $Ca_V3.1$, $Ca_V3.2$ and $Ca_V3.3$ mRNA in the rat nervous system has been extensively examined using *in situ* hybridization (9, 10). Other studies have examined the distribution of the T-type mRNA isoforms in the human brain via Northern blotting experiments (7, 8, 11). There was wide agreement among these studies in the patterns of distribution and it was shown that many brain regions express more than one type of Ca_V3 mRNA. $Ca_V3.1$ mRNA was abundant in many brain regions, especially the thalamic relay nuclei, cerebellum, amygdala, hippocampus, olfactory bulb, cerebral cortex and claustrum (9, 10). Another study found $Ca_V3.1$-like immunoreactivity of similar distribution in the rat brain and noted that it was usually localized in both the dendrites and soma of neurons (12). The highest expression of $Ca_V3.2$ mRNA in the brain was found in the olfactory bulb, indusium griseum and hippocampus, whereas $Ca_V3.3$ mRNA was most apparent in the olfactory bulb, cerebral cortex, hippocampus and thalamus (9).

In the spinal cord, $Ca_V3.1$, $Ca_V3.2$ and $Ca_V3.3$ mRNA expression was present in the dorsal horn at different levels (9); $Ca_V3.1$ mRNA had a moderate and even expression throughout, $Ca_V3.2$ mRNA was also moderately expressed yet restricted to the superficial dorsal horn, and $Ca_V3.3$ mRNA expression was low and most prominent in laminae III and IV. $Ca_V3.1$ and $Ca_V3.2$ mRNA was also found in the ventral horn of the spinal cord at low to moderate levels (9). Others reported a higher level of staining for $Ca_V3.1$ mRNA in the ventral horn compared to the dorsal horn of the spinal cord (10). In contrast to its expression in the spinal cord, $Ca_V3.1$ mRNA is undetectable in dorsal root ganglia (DRG) (9, 13). Talley *et al.* (1999) found a high level of $Ca_V3.2$ mRNA and moderate expression of $Ca_V3.3$ mRNA in small and medium-sized rat DRG cells. However, others have reported a similar degree of expression of $Ca_V3.3$ mRNA in rat DRG cells of all sizes (13). Interestingly, it has been reported that $Ca_V3.2$ expression in the mouse DRG is specific to one type of mechanoreceptor, the very sensitive D-hair receptor (14).

Physiology of T-type calcium channels

As previously mentioned, T-type calcium channels acquired their name due to their transient current during a sustained pulse and their tiny single-channel conductance of Ca^{2+} and Ba^{2+} ions. Other major characteristics of T-type channels have been established through electrophysiological experiments and include: channel opening after small membrane depolarizations, *i.e.*, they are LVA and slow deactivating tail currents due to slow channel closing when the membrane is repolarized (for an extensive review on T-type current electrophysiology, see Ref. 15). In DRG neurons, T-type currents are observed at membrane potentials of –60 mV, a much lower activa-

tion threshold than the –30 mV membrane potential required to generate HVA calcium currents (1, 16). Complete steady-state inactivation of T-type channels arises at membrane potentials higher than –50 mV and full activation of T-type channels is seen when the membrane is hyperpolarized (–100 mV) (1). Of importance to nociceptive transmission, T-type currents have been recorded in small and medium-sized DRG neurons (17, 18), superficial dorsal horn neurons (19) and thalamic neurons (20). Hence, these earlier electrophysiological studies parallel the later reports on the expression of T-type α1 subunit isoforms described in the previous section.

T-type calcium channels cannot sustain neurotransmission unaided (21) and do not mediate neurotransmitter release. They are involved in neuronal bursting activity and, due to their slow deactivation kinetics, contribute substantial calcium influx during membrane repolarization. The activation of T-type calcium channels in neurons generates low-threshold calcium spikes that can trigger bursts of action potentials, which can promote neuronal hyperexcitability (see below). Their preferential localization on dendrites underlies their role in enhancing synaptic signals. T-type calcium channels play a key role in the oscillatory behavior of thalamic neurons, which transmit information, often in a burst firing pattern, to the cerebral cortex (15).

The cloning of the T-type calcium channel α1 subunit gene family enabled further investigation into the kinetics of T-type calcium currents. When $Ca_v3.1$, $Ca_v3.2$ and $Ca_v3.3$ isoforms are expressed in *Xenopus* oocytes or mammalian cells, hallmark low-threshold-activated, transient and slowly deactivating calcium currents are observed, akin to those previously recorded in animal tissues. Of the three isoforms, $Ca_v3.1$ currents have the fastest activation and inactivation kinetics and $Ca_v3.3$ currents the slowest (5-fold slower than $Ca_v3.1$ currents) (22).

Alternative splicing has also been implicated in the functional properties of T-type calcium channels. Splice variants of human brain $Ca_v3.3$ channels had a differential expression across the brain (23) and whole-cell patch clamp recording demonstrated different activation and inactivation kinetics for these splice variants (24). Using $Ca_v3.1$ knockout mice, $Ca_v3.1$ channels were shown to be essential in thalamic burst firing (25). In addition, the specific contribution of the different human calcium channel isoforms to neuronal excitability has been examined and results indicated that $Ca_v3.1$ and $Ca_v3.2$ currents generate short burst firing, whereas $Ca_v3.3$ current contribute to persistent electrical activity (26). There are currently no pharmacological agents which can distinguish between the different T-type calcium channel isoforms.

Evidence for a role for T-type calcium channels in pain

The effect of nerve injury on T-type calcium currents in DRG has been examined in several studies. In two studies from different laboratories, electrophysiological recordings from DRG T-type calcium currents 2-7 weeks following complete sciatic nerve transection (axotomy) showed that these currents were unaffected by this neuronal insult (27, 28). In contrast to these results, a loss of T-type calcium currents in medium-sized DRG cells was seen following axotomy (29) and chronic constriction injury (CCI) to the sciatic nerve (30). In addition, the loss of T-type current in CCI rats was accompanied by an increase in neuronal excitability of DRG cells (31). This increase in neuronal excitability could be explained by the decreased calcium influx, resulting in a decrease in the inhibitory calcium-activated potassium current. T-type calcium currents in thalamic relay neurons were increased by 68% and 44% at 1 and 3 days, respectively, following unilateral corticectomy (32). Thus, the effect of nerve injury on T-type calcium currents appears to be dependent upon the site of the nerve injury.

An extensive electrophysiological study presented evidence that T-type calcium channels play a direct role in the mechanisms of hyperalgesia (33). A subset of lamina I spinal neurons which express NK_1 receptors have been shown to mediate hyperalgesia, as their ablation by a substance P-conjugated toxin attenuated inflammation- or nerve injury-evoked hyperalgesia (34, 35). The induction of long-term potentiation (LTP) in lamina I spinal neurons by conditioning C-fiber stimulation is an event which underlies hyperalgesia in pain states and can be experimentally replicated. It was elegantly demonstrated that the induction of LTP is dependent upon the coactivation of not only NK_1 and NMDA receptors, but also T-type calcium currents (33).

Peripheral T-type calcium channels have been shown to be important in the generation of pain through redox modulation. L-Cysteine and dithiothreitol (DTT) are reducing agents which enhance T-type calcium currents in rat DRG neurons and recombinant $Ca_v3.2$ channels (36). Furthermore, injection of these reducing agents into the paws of normal rats induced thermal hyperalgesia that was reversed by the oxidizing agent 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB) and the T-type blocker mibefradil (36). Such experiments were repeated in nerve-injured (CCI) rats and it was found that L-cysteine enhanced and DTNB inhibited thermal hyperalgesia evoked by nerve injury (37).

The role of T-type calcium channels in pain has also been addressed using specific genetic modulation of T-type calcium channel isoforms. $Ca_v3.1$ knockout mice were shown to have an increased sensitivity to visceral pain, but no difference in acute pain thresholds or responses to a cutaneous pain stimulus compared to wild-type mice (38). Electrophysiological recordings from ventroposterolateral (VPL) thalamocortical neurons showed no effect of the significant genetic mutation on the firing rate of single spikes, but a depletion of burst firing in $Ca_v3.1$ knockout mice compared to wild-type mice (38). This study suggests that: 1) somatic nociception is processed differently to visceral pain; and 2) VPL thalamocortical neurons are activated initially in response to a

surge of visceral input and then provide an inhibitory function in processing visceral nociceptive signals. Recently, the effects of intrathecally administered oligodeoxynucleotide antisense compounds specific for $Ca_V3.1$, $Ca_V3.2$ and $Ca_V3.3$ were examined in normal and nerve-injured (CCI) rats (39). $Ca_V3.2$ antisense knockdown reduced T-type currents in small and medium-sized DRG cells. Acute mechanical and thermal responses were markedly increased in normal rats. Moreover, a near complete reversal of mechanical allodynia in CCI rats was observed and considerable inhibition of heat and mechanical hyperalgesia, such that these responses were of similar magnitude to the responses of normal rats treated with $Ca_V3.2$. Together, the results of these two studies (38, 39) suggest that the different T-type calcium channel isoforms can be antinociceptive or pronociceptive. $Ca_V3.1$ is antinociceptive, extensively expressed in the thalamus, but absent in the DRG (9). Thus, intraperitoneal administration of mibefradil, a T-type blocker, diminished visceral pain (38). In contrast, $Ca_V3.2$ is pronociceptive, expressed in the DRG, but absent in the thalamus (9). Thus, intrathalamic administration enhanced visceral pain (38).

Perhaps the major problem with elucidating the exact role of T-type calcium channels in chronic pain is the current lack of highly specific pharmacological agents. At present, there are a handful of drugs which produce a relatively selective blockade of T-type calcium channels. An up-to-date summary of their *in vivo* analgesic effects both in the laboratory and the clinic is discussed below.

Ethosuximide (Zarontin®)

Ethosuximide is a succinimide derivative that was introduced in the U.S. in 1960 for the treatment of petit mal (absence) epilepsy, and it still remains the drug of choice for this condition (40). Ethosuximide blocks cloned human T-type calcium channels in a state-dependent manner, having a higher affinity for inactivated channels (41). It is relatively selective for T-type calcium channels, blocking T-type currents at a concentration 2-fold less than that required for L-type current blockade in rat DRG neurons (42). The antiepileptic properties of ethosuximide were originally thought to be due to blockade of T-type currents in thalamic neurons (20). However, ethosuximide has also been shown to inhibit certain sodium and potassium currents in thalamic neurons (43). Thus, there is a possibility that the effects of ethosuximide may occur as a result of a blockade at several types of ion channels.

Ethosuximide has been tested *in vivo* in a number of laboratory pain paradigms. When administered into the paw of normal rats, a short-lasting antinociceptive effect was observed by the measurement of thermal withdrawal latencies (44). Spinal administration of ethosuximide inhibited the responses of spinal (wide-dynamic range) neurons in a similar manner, both before and after nerve injury (45). Ethosuximide has been shown to inhibit both hyperalgesia and edema evoked by the inflammatory agents carrageenan and complete Freund's adjuvant (46).

The analgesic effect of ethosuximide has also been demonstrated in a number of rat models of neuropathic pain. Systemic (i.p.) ethosuximide dose-dependently reversed mechanical, heat and cold hypersensitivity evoked by peripheral nerve injury, either CCI or spinal nerve ligation (SNL) (46, 47). However, intrathecal ethosuximide did not inhibit SNL-evoked mechanical and heat hypersensitivity (47), which could suggest an insufficient dose was administered or that the analgesic effect of ethosuximide is exerted via actions at peripheral T-type channels. However, intraplantar ethosuximide administration also failed to inhibit mechanical allodynia (47). This could be an indication of the poor solubility of the drug, or its limited potency, as intraplantar mibefradil was effective in this experimental setting, as discussed below. In addition, ethosuximide produced a dose-related, marked reversal of mechanical allodynia/hyperalgesia, without tolerance, in rats with chemotherapy-induced (paclitaxel or vincristine) painful peripheral neuropathy (48). This study also demonstrated that ethosuximide could reverse paclitaxel-induced cold allodynia (48).

The systemic doses used in these rat studies are within the dosing range effective in rat models of epilepsy. Unlike gabapentin, whose analgesic efficacy was initially identified in adult epileptic patients who also had neuropathic pain, there have been no such translation cases with ethosuximide use. The reason for this is likely to be that ethosuximide is a treatment for petit mal epilepsy, a condition that is predominantly seen in children and usually dissipates by adulthood. Unfortunately, there have been no clinical trials, either open-label or randomized, with ethosuximide in chronic pain patients. The only report on ethosuximide in a clinical pain setting describes 3 patients with migraine who were unresponsive to standard therapies but achieved remarkable relief of their symptoms with ethosuximide therapy (49).

Mibefradil (Posicor®)

Mibefradil is a novel tetrazole selected for development by Roche from over 500 analogues of the phenylalkylamine verapamil, an L-type calcium channel antagonist. Mibefradil was marketed as Posicor® for the treatment of stable angina pectoris and hypertension, but was subsequently withdrawn from the market because it was found to inhibit certain cytochrome P-450 enzymes which resulted in the dangerous accumulation of numerous other drugs that are normally eliminated via hepatic mechanisms. Of the drugs with T-type calcium channel-blocking capability, mibefradil is currently the most selective agent available. Mibefradil is 10-50-fold more selective for T-type calcium channels compared to L-type calcium channels, depending on the experimental scenario (50-52). Mibefradil appears to be equally potent at all three cloned T-type calcium channels (53), although there is evidence to suggest that it is 2-fold less selective

for $Ca_V3.3$ (8). Like ethosuximide, mibefradil has also been shown to block a variety of other ion channels *in vitro,* including sodium channels, potassium channels and N-, P/Q- and L-type calcium channels (for a review of these experiments, see Ref. 54).

Systemic (i.p.) mibefradil has been shown to exert analgesic activity in normal rats, increasing responses to mechanical and heat stimuli in a dose-related manner (55). Intraperitoneal mibefradil also dose-dependently inhibited thermal and mechanical hypersensitivity evoked by nerve injury (SNL) in rats (47). As observed with intrathecal ethosuximide, intrathecal mibefradil administration also had no effect on mechanical allodynia and heat hyperalgesia in SNL rats (47). Unlike ethosuximide, however, intraplantar mibefradil inhibited SNL-evoked mechanical and heat hypersensitivity (47), indicating potentially greater potency for mibefradil at peripheral T-type calcium channels. There are no reports on the clinical efficacy of mibefradil in chronic pain patients, and considering its withdrawal from the market for safety reasons, this is unlikely to change.

Zonisamide (Zonegran®)

Zonisamide is a relatively new antiepileptic drug derived from sulfonamide. It was initially introduced in Japan, followed several years later, in 2000, by launch in the U.S. It has been shown to block T-type calcium channels in the rat cerebral cortex (56) and in human neuroblastoma cells (57), and it also blocks voltage-dependent sodium channels (58). Other potential mechanisms of action for zonisamide have been reported, including effects on dopaminergic transmission (59), serotonergic transmission (60), free radicals (61) and nitric oxide (62). The dominant mechanism of action of zonisamide is not yet clear. Nevertheless, zonisamide significantly reduced thermal hyperalgesia and, to a lesser extent, mechanical allodynia in neuropathic rats (63).

To date, no randomized, controlled clinical trials have been performed with zonisamide in neuropathic pain patients. However, there are reports of open-label trials and case reports on its use in chronic pain patients. Zonisamide was given as adjunctive therapy to 40 patients who had treatment-refractory neuropathic pain due to cervical or lumbar radiculopathies (64). Ten patients reported a decrease of > 60% and another 8 patients a 30-60% decrease in their daily pain scores. A retrospective review of neuropathic pain patients at an outpatient clinic found that 100 of 142 patients reported moderate pain relief with zonisamide (64). Two patients with severe intractable central poststroke pain received substantial pain relief with zonisamide (65). In another case report, a patient with idiopathic polyneuropathy who had responded poorly to a variety of pharmacotherapies experienced a decrease in the pain score (scale of 0-10) from 9 at baseline to 5 following zonisamide therapy (66).

Phenytoin (Dilantin®)

Phenytoin is an old antiepileptic drug which has been available since the 1950s. It was initially described as a sodium channel blocker, but it can also block T-type calcium channels at therapeutic concentrations (18, 67). Phenytoin inhibits T-type currents in native rat DRG neurons (18), neuroblastoma cells (67), rat hippocampal neurons (68) and rat thalamic neurons (20). Electrophysiological experiments with cells expressing cloned T-type calcium channel isoforms demonstrated that phenytoin elicited a complete, low-affinity block of $Ca_V3.1$ and a mixed-sensitivity/affinity block of $Ca_V3.2$ (69).

Phenytoin inhibits spontaneous (ectopic) firing in neuromas of rat sciatic nerves (70). When administered into the paws of normal rats, phenytoin produced a dose-related inhibition of thermal nociception (44). However, it was ineffective at inhibiting mechanical and heat hyperalgesia evoked by two types of peripheral nerve injury (CCI and SNL) (71). Interestingly, phenytoin provided dose-dependent relief from pain evoked by bradykinin application to tooth pulp in rats (72). The results of this study are in agreement with the traditional use of phenytoin in the treatment of trigeminal neuralgia, although no clinical trials have been performed in this condition.

The clinical experience with phenytoin as a treatment for chronic pain has been varied. Two randomized, double-blind, placebo-controlled trials of phenytoin in diabetic neuropathy were reported (73, 74); one found it to be more effective than placebo (73), while the other found it to be ineffective (74). Mixed effects of phenytoin were reported in 8 patients with thalamic pain, some reporting beneficial effects and others detrimental effects (75). A small randomized, double-blind trial of phenytoin in cancer pain patients concluded that it produced mild to moderate relief of cancer pain and significantly enhanced buprenorphine analgesia (76). Lastly, there is evidence that intravenous phenytoin may prove beneficial in patients who experience acute attacks of neuropathic pain. This was demonstrated in a randomized, double-blind, placebo-controlled trial in patients with acute neuropathic pain flare-ups caused by various neuropathies (77), and also in a patient with crescendo pelvic cancer-related pain (78).

T-type calcium channel blockers – analgesic drugs of the future?

The answer to this question is dependent on the generation of more selective T-type calcium channel blockers. A number of agents have shown selectivity for T-type calcium channels in electrophysiological experiments. Kurtoxin is a scorpion toxin that binds with high affinity to $Ca_V3.1$ channels (79). Certain derivatives of piperazinyl-alkylisoxazole (80) and 3,4-dihydroquinazoline (81) have been reported to be potent T-type channel blockers. In addition, ω-3 fatty acids have been shown to be relatively selective at blocking native T-type calcium

channels (82). Neuroactive steroids have also been reported to inhibit acute nociception following peripheral administration (83, 84). Perhaps further experiments with these compounds in animal models of chronic pain will shed more light on the contribution of T-type calcium channels in chronic pain. Such efforts, in concert with the generation of a truly selective T-type calcium channel blocker, would validate whether the T-type calcium channel is a superior target for analgesic development.

References

1. Nowycky, M.C., Fox, A.P., Tsien, R.W. *Three types of neuronal calcium channel with different calcium agonist sensitivity*. Nature 1985, 316(6027): 440-3.

2. Walker, D., De Waard, M. *Subunit interaction sites in voltage-dependent Ca^{2+} channels: Role in channel function*. Trends Neurosci 1998, 21(4): 148-54.

3. Ertel, E.A., Campbell, K.P., Harpold, M.M. et al. *Nomenclature of voltage-gated calcium channels*. Neuron 2000, 25(3): 533-5.

4. Perez-Reyes, E., Cribbs, L.L., Daud, A. et al. *Molecular characterization of a neuronal low-voltage-activated T-type calcium channel*. Nature 1998, 391(6670): 896-900.

5. Cribbs, L.L., Lee, J.H., Yang, J. et al. *Cloning and characterization of α1H from human heart, a member of the T-type Ca^{2+} channel gene family*. Circ Res 1998, 83(1): 103-9.

6. Lee, J.H., Daud, A.N., Cribbs, L.L. et al. *Cloning and expression of a novel member of the low voltage-activated T-type calcium channel family*. J Neurosci 1999, 19(6): 1912-21.

7. Monteil, A., Chemin, J., Bourinet, E., Mennessier, G., Lory, P., Nargeot, J. *Molecular and functional properties of the human α(1G) subunit that forms T-type calcium channels*. J Biol Chem 2000, 275(9): 6090-100.

8. Monteil, A., Chemin, J., Leuranguer, V. et al. *Specific properties of T-type calcium channels generated by the human α 1I subunit*. J Biol Chem 2000, 275(22): 16530-5.

9. Talley, E.M., Cribbs, L.L., Lee, J.H., Daud, A., Perez-Reyes, E., Bayliss, D.A. *Differential distribution of three members of a gene family encoding low voltage-activated (T-type) calcium channels*. J Neurosci 1999, 19(6): 1895-911.

10. Kase, M., Kakimoto, S., Sakuma, S. et al. *Distribution of neurons expressing α 1G subunit mRNA of T-type voltage-dependent calcium channel in adult rat central nervous system*. Neurosci Lett 1999, 268(2): 77-80.

11. Williams, M.E., Washburn, M.S., Hans, M. et al. *Structure and functional characterization of a novel human low-voltage activated calcium channel*. J Neurochem 1999, 72(2): 791-9.

12. Craig, P.J., Beattie, R.E., Folly, E.A. et al. *Distribution of the voltage-dependent calcium channel α1G subunit mRNA and protein throughout the mature rat brain*. Eur J Neurosci 1999, 11(8): 2949-64.

13. Yusaf, S.P., Goodman, J., Pinnock, R.D., Dixon, A.K., Lee, K. *Expression of voltage-gated calcium channel subunits in rat dorsal root ganglion neurons*. Neurosci Lett 2001, 311(2): 137-41.

14. Shin, J.B., Martinez-Salgado, C., Heppenstall, P.A., Lewin, G.R. *A T-type calcium channel required for normal function of a mammalian mechanoreceptor*. Nat Neurosci 2003, 6(7): 724-30.

15. Perez-Reyes, E. *Molecular physiology of low-voltage-activated T-type calcium channels*. Physiol Rev 2003, 83(1): 117-61.

16. Fedulova, S.A., Kostyuk, P.G., Veselovsky, N.S. *Two types of calcium channels in the somatic membrane of new-born rat dorsal root ganglion neurones*. J Physiol 1985, 359: 431-46.

17. Scroggs, R.S., Fox, A.P. *Calcium current variation between acutely isolated adult rat dorsal root ganglion neurons of different size*. J Physiol 1992, 445: 639-58.

18. Todorovic, S.M., Lingle, C.J. *Pharmacological properties of T-type Ca^{2+} current in adult rat sensory neurons: Effects of anticonvulsant and anesthetic agents*. J Neurophysiol 1998, 79(1): 240-52.

19. Ryu, P.D., Randic, M. *Low- and high-voltage-activated calcium currents in rat spinal dorsal horn neurons*. J Neurophysiol 1990, 63(2): 273-85.

20. Coulter, D.A., Huguenard, J.R., Prince, D.A. *Characterization of ethosuximide reduction of low-threshold calcium current in thalamic neurons*. Ann Neurol 1989, 25(6): 582-93.

21. Pfrieger, F.W., Veselovsky, N.S., Gottmann, K., Lux, H.D. *Pharmacological characterization of calcium currents and synaptic transmission between thalamic neurons in vitro*. J Neurosci 1992, 12(11): 4347-57.

22. Klockner, U., Lee, J.H., Cribbs, L.L. et al. *Comparison of the Ca^{2+} currents induced by expression of three cloned α1 subunits, α1G, α1H and α1I, of low-voltage-activated T-type Ca^{2+} channels*. Eur J Neurosci 1999, 11(12): 4171-8.

23. Murbartian, J., Arias, J.M., Lee, J.H., Gomora, J.C., Perez-Reyes, E. *Alternative splicing of the rat $Ca_V3.3$ T-type calcium channel gene produces variants with distinct functional properties(1)*. FEBS Lett 2002, 528(1-3): 272-8.

24. Murbartian, J., Arias, J.M., Perez-Reyes, E. *Functional impact of alternative splicing of human T-type Cav3.3 calcium channels*. J Neurophysiol 2004, 92(6): 3399-407.

25. Kim, D., Song, I., Keum, S. et al. *Lack of the burst firing of thalamocortical relay neurons and resistance to absence seizures in mice lacking α(1G) T-type Ca^{2+} channels*. Neuron 2001, 31(1): 35-45.

26. Chemin, J., Monteil, A., Perez-Reyes, E., Bourinet, E., Nargeot, J., Lory, P. *Specific contribution of human T-type calcium channel isotypes (α(1G), α(1H) and α(1I)) to neuronal excitability*. J Physiol 2002, 540(Pt. 1): 3-14.

27. Baccei, M.L., Kocsis, J.D. *Voltage-gated calcium currents in axotomized adult rat cutaneous afferent neurons*. J Neurophysiol 2000, 83(4): 2227-38.

28. Abdulla, F.A., Smith, P.A. *Axotomy- and autotomy-induced changes in Ca^{2+} and K^+ channel currents of rat dorsal root ganglion neurons*. J Neurophysiol 2001, 85(2): 644-58.

29. Andre, S., Puech-Mallie, S., Desmadryl, G., Valmier, J., Scamps, F. *Axotomy differentially regulates voltage-gated calcium currents in mice sensory neurones*. Neuroreport 2003, 14(1): 147-50.

30. Hogan, Q.H., McCallum, J.B., Sarantopoulos, C. et al. *Painful neuropathy decreases membrane calcium current in mammalian primary afferent neurons*. Pain 2000, 86(1-2): 43-53.

31. McCallum, J.B., Kwok, W.M., Mynlieff, M., Bosnjak, Z.J., Hogan, Q.H. *Loss of T-type calcium current in sensory neurons of rats with neuropathic pain*. Anesthesiology 2003, 98(1): 209-16.

32. Chung, J.M., Huguenard, J.R., Prince, D.A. *Transient enhancement of low-threshold calcium current in thalamic relay neurons after corticectomy*. J Neurophysiol 1993, 70(1): 20-7.

33. Ikeda, H., Heinke, B., Ruscheweyh, R., Sandkuhler, J. *Synaptic plasticity in spinal lamina I projection neurons that mediate hyperalgesia*. Science 2003, 299(5610): 1237-40.

34. Mantyh, P.W., Rogers, S.D., Honore, P. et al. *Inhibition of hyperalgesia by ablation of lamina I spinal neurons expressing the substance P receptor*. Science 1997, 278(5336): 275-9.

35. Nichols, M.L., Allen, B.J., Rogers, S.D. et al. *Transmission of chronic nociception by spinal neurons expressing the substance P receptor*. Science 1999, 286(5444): 1558-61.

36. Todorovic, S.M., Jevtovic-Todorovic, V., Meyenburg, A. et al. *Redox modulation of T-type calcium channels in rat peripheral nociceptors*. Neuron 2001, 31(1): 75-85.

37. Todorovic, S.M., Meyenburg, A., Jevtovic-Todorovic, V. *Redox modulation of peripheral T-type Ca^{2+} channels in vivo: Alteration of nerve injury-induced thermal hyperalgesia*. Pain 2004, 109(3): 328-39.

38. Kim, D., Park, D., Choi, S. et al. *Thalamic control of visceral nociception mediated by T-type Ca^{2+} channels*. Science 2003, 302(5642): 117-9.

39. Bourinet, E., Alloui, A., Monteil, A. et al. *Silencing of the $Ca_v3.2$ T-type calcium channel gene in sensory neurons demonstrates its major role in nociception*. EMBO J 2005, 24(2): 315-24.

40. Mattson, R.H. *Efficacy and adverse effects of established and new antiepileptic drugs*. Epilepsia 1995, 36(Suppl. 2): S13-26.

41. Gomora, J.C., Daud, A.N., Weiergraber, M., Perez-Reyes, E. *Block of cloned human T-type calcium channels by succinimide antiepileptic drugs*. Mol Pharmacol 2001, 60(5): 1121-32.

42. Kostyuk, P.G., Molokanova, E.A., Pronchuk, N.F., Savchenko, A.N., Verkhratsky, A.N. *Different action of ethosuximide on low- and high-threshold calcium currents in rat sensory neurons*. Neuroscience 1992, 51(4): 755-8.

43. Leresche, N., Parri, H.R., Erdemli, G. et al. *On the action of the anti-absence drug ethosuximide in the rat and cat thalamus*. J Neurosci 1998, 18(13): 4842-53.

44. Todorovic, S.M., Rastogi, A.J., Jevtovic-Todorovic, V. *Potent analgesic effects of anticonvulsants on peripheral thermal nociception in rats*. Br J Pharmacol 2003, 140(2): 255-60.

45. Matthews, E.A., Dickenson, A.H. *Effects of ethosuximide, a T-type Ca^{2+} channel blocker, on dorsal horn neuronal responses in rats*. Eur J Pharmacol 2001, 415(2-3): 141-9.

46. Gogas, K.R., Loo, C., Lewis, R. et al. *Effects of the T-type calcium channel blocker ethosuximide in rodent models of acute and chronic pain.* 10th World Cong Pain (Seattle) 2002, 275.

47. Dogrul, A., Gardell, L.R., Ossipov, M.H., Tulunay, F.C., Lai, J., Porreca, F. *Reversal of experimental neuropathic pain by T-type calcium channel blockers*. Pain 2003, 105(1-2): 159-68.

48. Flatters, S.J.L., Bennett, G.J. *Ethosuximide reverses paclitaxel- and vincristine-induced painful peripheral neuropathy*. Pain 2004, 109(1-2): 150-61.

49. Eggers, A.E., Moreno, H. *Suggestion for another anti-epileptic drug to use for migraine prophylaxis*. Headache 2002, 42(7): 696-7.

50. Mishra, S.K., Hermsmeyer, K. *Selective inhibition of T-type Ca^{2+} channels by Ro 40-5967*. Circ Res 1994, 75(1): 144-8.

51. Jimenez, C., Bourinet, E., Leuranguer, V., Richard, S., Snutch, T.P., Nargeot, J. *Determinants of voltage-dependent inactivation affect mibefradil block of calcium channels*. Neuropharmacology 2000, 39(1): 1-10.

52. Clozel, J.P., Ertel, E.A., Ertel, S.I. *Voltage-gated T-type Ca^{2+} channels and heart failure*. Proc Assoc Am Physic 1999, 111(5): 429-37.

53. Martin, R.L., Lee, J.H., Cribbs, L.L., Perez-Reyes, E., Hanck, D.A. *Mibefradil block of cloned T-type calcium channels*. J Pharmacol Exp Ther 2000, 295(1): 302-8.

54. Heady, T.N., Gomora, J.C., Macdonald, T.L., Perez-Reyes, E. *Molecular pharmacology of T-type Ca^{2+} channels*. Jpn J Pharmacol 2001, 85(4): 339-50.

55. Todorovic, S.M., Meyenburg, A., Jevtovic-Todorovic, V. *Mechanical and thermal antinociception in rats following systemic administration of mibefradil, a T-type calcium channel blocker*. Brain Res 2002, 951(2): 336-40.

56. Suzuki, S., Kawakami, K., Nishimura, S. et al. *Zonisamide blocks T-type calcium channel in cultured neurons of rat cerebral cortex*. Epilepsy Res 1992, 12(1): 21-7.

57. Kito, M., Maehara, M., Watanabe, K. *Mechanisms of T-type calcium channel blockade by zonisamide*. Seizure 1996, 5(2): 115-9.

58. Sobieszek, G., Borowicz, K.K., Kimber-Trojnar, Z., Malek, R., Piskorska, B., Czuczwar, S.J. *Zonisamide: A new antiepileptic drug*. Pol J Pharmacol 2003, 55(5): 683-9.

59. Okada, M., Kaneko, S., Hirano, T. et al. *Effects of zonisamide on dopaminergic system*. Epilepsy Res 1995, 22(3): 193-205.

60. Okada, M., Hirano, T., Kawata, Y. et al. *Biphasic effects of zonisamide on serotonergic system in rat hippocampus*. Epilepsy Res 1999, 34(2-3): 187-97.

61. Mori, A., Noda, Y., Packer, L. *The anticonvulsant zonisamide scavenges free radicals*. Epilepsy Res 1998, 30(2): 153-8.

62. Noda, Y., Mori, A., Packer, L. *Zonisamide inhibits nitric oxide synthase activity induced by N-methyl-D-aspartate and buthionine sulfoximine in the rat hippocampus*. Res Commun Mol Pathol Pharmacol 1999, 105(1-2): 23-33.

63. Hord, A.H., Denson, D.D., Chalfoun, A.G., Azevedo, M.I. *The effect of systemic zonisamide (Zonegran) on thermal hyperalgesia and mechanical allodynia in rats with an experimental mononeuropathy*. Anesth Analg 2003, 96(6): 1700-6.

64. Pappagallo, M. *Newer antiepileptic drugs: Possible uses in the treatment of neuropathic pain and migraine*. Clin Ther 2003, 25(10): 2506-38.

65. Takahashi, Y., Hashimoto, K., Tsuji, S. *Successful use of zonisamide for central poststroke pain*. J Pain 2004, 5(3): 192-4.

66. Bernstein, C., Diaz, J., Gould, H. *A possible role for zonisamide in treating neuropathic pain: A case of idiopathic polyneuropathy*. Pain Practise 2002, 2: 134-6.

67. Twombly, D.A., Yoshii, M., Narahashi, T. *Mechanisms of calcium channel block by phenytoin*. J Pharmacol Exp Ther 1988, 246(1): 189-95.

68. Yaari, Y., Hamon, B., Lux, H.D. *Development of two types of calcium channels in cultured mammalian hippocampal neurons*. Science 1987, 235(4789): 680-2.

69. Todorovic, S.M., Perez-Reyes, E., Lingle, C.J. *Anticonvulsants but not general anesthetics have differential blocking effects on different T-type current variants*. Mol Pharmacol 2000, 58(1): 98-108.

70. Yaari, Y., Devor, M. *Phenytoin suppresses spontaneous ectopic discharge in rat sciatic nerve neuromas*. Neurosci Lett 1985, 58(1): 117-22.

71. Hunter, J.C., Gogas, K.R., Hedley, L.R. et al. *The effect of novel anti-epileptic drugs in rat experimental models of acute and chronic pain*. Eur J Pharmacol 1997, 324(2-3): 153-60.

72. Foong, F.W., Satoh, M., Takagi, H. *A newly devised reliable method for evaluating analgesic potencies of drugs on trigeminal pain*. J Pharmacol Meth 1982, 7(4): 271-8.

73. Chadda, V.S., Mathur, M.S. *Double blind study of the effects of diphenylhydantoin sodium on diabetic neuropathy*. J Assoc Physic India 1978, 26(5): 403-6.

74. Saudek, C.D., Werns, S., Reidenberg, M.M. *Phenytoin in the treatment of diabetic symmetrical polyneuropathy*. Clin Pharmacol Ther 1977, 22(2): 196-9.

75. Agnew, D.C., Goldberg, V.D. *A brief trial of phenytoin therapy for thalamic pain*. Bull Los Angeles Neurol Soc 1976, 41(1): 9-12.

76. Yajnik, S., Singh, G.P., Singh, G., Kumar, M. *Phenytoin as a coanalgesic in cancer pain*. J Pain Symptom Manage 1992, 7(4): 209-13.

77. McCleane, G.J. *Intravenous infusion of phenytoin relieves neuropathic pain: A randomized, double-blinded, placebo-controlled, crossover study*. Anesth Analg 1999, 89(4): 985-8.

78. Chang, V.T. *Intravenous phenytoin in the management of crescendo pelvic cancer-related pain*. J Pain Symptom Manage 1997, 13(4): 238-40.

79. Chuang, R.S., Jaffe, H., Cribbs, L., Perez-Reyes, E., Swartz, K.J. *Inhibition of T-type voltage-gated calcium channels by a new scorpion toxin*. Nat Neurosci 1998, 1(8): 668-74.

80. Jung, H.K., Doddareddy, M.R., Cha, J.H. et al. *Synthesis and biological evaluation of novel T-type Ca^{2+} channel blockers*. Bioorg Med Chem 2004, 12(15): 3965-70.

81. Rhim, H., Lee, Y.S., Park, S.J., Chung, B.Y., Lee, J.Y. *Synthesis and biological activity of 3,4-dihydroquinazolines for selective T-type Ca^{2+} channel blockers*. Bioorg Med Chem Lett 2005, 15(2): 283-6.

82. Danthi, S.J., Enyeart, J.A., Enyeart, J.J. *Modulation of native T-type calcium channels by ω-3 fatty acids*. Biochem Biophys Res Commun 2005, 327(2): 485-93.

83. Todorovic, S.M., Pathirathna, S., Brimelow, B.C. et al. *5β-Reduced neuroactive steroids are novel voltage-dependent blockers of T-type Ca^{2+} channels in rat sensory neurons in vitro and potent peripheral analgesics in vivo*. Mol Pharmacol 2004, 66(5): 1223-35.

84. Pathirathna, S., Brimelow, B.C., Jagodic, M.M. et al. *New evidence that both T-type calcium channels and $GABA_A$ channels are responsible for the potent peripheral analgesic effects of 5α-reduced neuroactive steroids*. Pain 2005, 114(3): 429-43.